AGILENT ASSAYMAP SOLUTION FOR PHOSPHOPEPTIDE ENRICHMENT

Highly reproducible Phosphopeptide Enrichment with Fe(III)-NTA

Highlights

- Excellent reproducibility and phosphopeptide selectivity

- Powerful enrichment – recover sample in 10 µL of eluate

- Walkaway automation

- High-capacity Fe(III)-NTA resin with > 100 nmol Fe(III) per cartridge

Automate your phosphopeptide sample prep for LC/MS

AssayMAP Phosphopeptide Enrichment from Agilent Technologies is built on the powerful combination of miniaturized, packed-bed chromatography, the state-of-the art Agilent Bravo Automated Liquid Handling Platform, and a simple applications-based user interface. The push-button, automated environment simplifies phosphopeptide enrichment for novices and experienced users alike.

With AssayMAP Phosphopeptide Enrichment, up to 96 samples can be processed in parallel using cartridges packed with nitrilotriacetic acid charged with iron, Fe(III)-NTA. The Phosphopeptide Enrichment application may be used as a standalone application or integrated with other AssayMAP applications such as In-Solution Digestion and Peptide Cleanup to automate and streamline LC/MS analysis workflows. AssayMAP workflows dramatically improve reproducibility while decreasing hands on time.
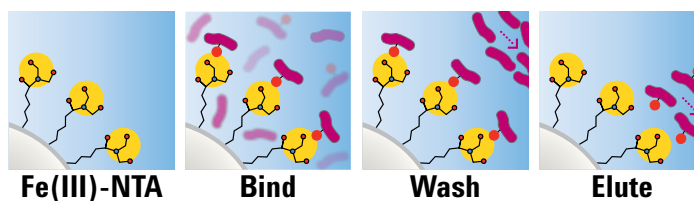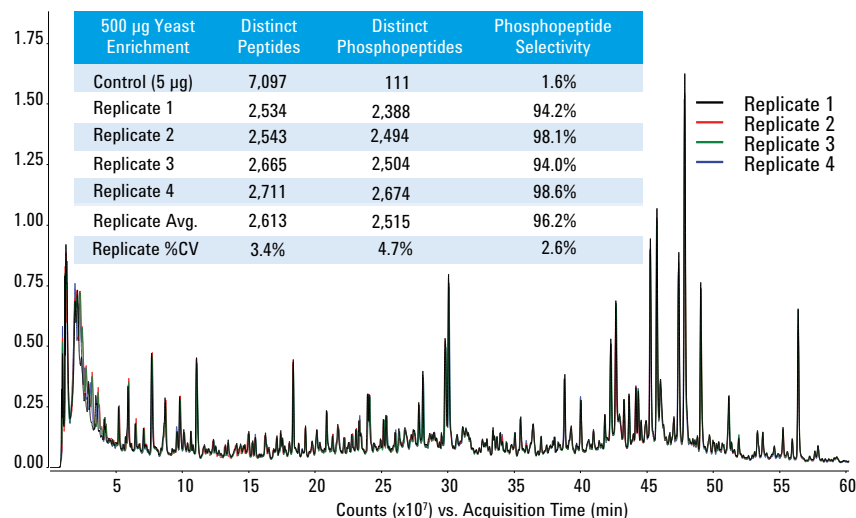

Fe(III)-NTA **Bind** **Wash** **Elute**

Figure 1. Phosphopeptide enrichment using AssayMAP Fe(III)-NTA cartridges

### Ordering information for AssayMAP Fe(III)-NTA cartridges

Product description	Part number	
AssayMAP Fe(III)-NTA Cartridge Rack	G5496-60085	1 rack of 96 cartridges

The Measure of Confidence

Agilent Technologies

500 μg Yeast Enrichment	Distinct Peptides	Distinct Phosphopeptides	Phosphopeptide Selectivity
Control (5 μg)	7,097	111	1.6%
Replicate 1	2,534	2,388	94.2%
Replicate 2	2,543	2,494	98.1%
Replicate 3	2,665	2,504	94.0%
Replicate 4	2,711	2,674	98.6%
Replicate Avg.	2,613	2,515	96.2%
Replicate %CV	3.4%	4.7%	2.6%

Unparalleled reproducibility and enrichment of phosphopeptides from trypsinized cell lysates.

Protein from yeast whole-cell lysate was extracted, bulk trypsinized, desalted, and lyophilized. An estimated 500-μg aliquot of reconstituted digest (80% ACN/0.1% TFA) was loaded onto Fe(III)-NTA cartridges in quadruplicate. Cartridges were washed with sample solvent, eluted with aqueous ammonia, and analyzed on a 6550 iFunnel Q-TOF equipped with a Dual AJS ESI source coupled to a 1290 infinity LC with a 2.1 x 150 mm AdvanceBio Peptide Mapping column. Samples were eluted at 400 μL/min over a 60-minute gradient (TIC overlays shown above). Acquired spectra were processed using Spectrum Mill with peptide identifications filtered to a 1.1% FDR. Excellent reproducibility was achieved at both the distinct peptide and phosphopeptide levels. The average phosphopeptide selectivity was greater than 96% yielding a 60-fold enrichment.
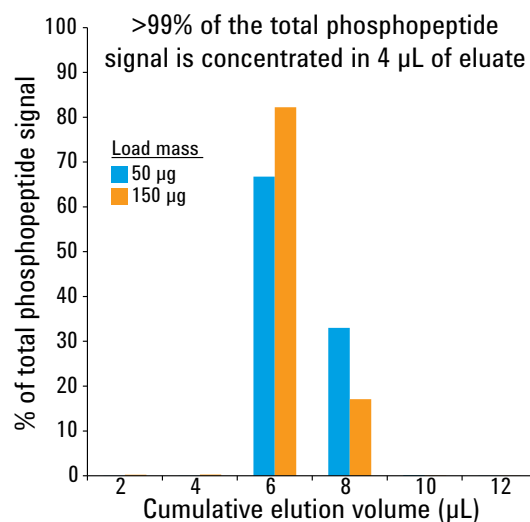
Phosphopeptides from tryptic digests of bovine α-casein were eluted from AssayMAP Fe(III)-NTA cartridges in 2-μL fractions using 1% aqueous ammonia and analyzed by LC/MS. Complete elution was achieved in 10 μL of elution buffer with more than 99% of the total phosphopeptide signal concentrated in 4 μL of eluate.

Recover your sample in extremely low volumes.

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